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Effects of Fenvalerate on Larval Development of 'Palaemonetes pugio' (Holthuis) and on Larval Metabolism during Osmotic Stress

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16. ABSTRACT

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Key words: fenvalerate, Palaemonetes pugio, larval development, oxygen consumption, osmotic stress, pyrethroid insecticides.

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EFFECTS OF FENVALERATE ON LARVAL DEVELOPMENT OF *PALAEMONETES PUGIO* (HOLTHUIS) AND ON LARVAL METABOLISM DURING OSMOTIC STRESS

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Larvae of the estuarine grass shrimp, *Palaemonetes pugio*, were reared in the laboratory from hatch through metamorphosis under optimal salinity conditions (20‰) in a range of lethal and sublethal concentrations of the pyrethroid insecticide, fenvalerate. A nominal concentration of 3.2 ng fenvalerate/l significantly reduced the percentage of larvae that completed metamorphosis. Oxygen consumption rates were significantly higher for larvae exposed to this lethal concentration for only 24 h. Exposure to a sublethal concentration of 1.6 ng fenvalerate/l prolonged the duration of complete larval development.

Acute osmotic stress modified the metabolism of larval *P. pugio* reared in sublethal fenvalerate concentrations of 0.1 and 0.2 ng/l and these metabolic responses varied with stage of development. After 8 days of exposure to fenvalerate, oxygen consumption rates were elevated when larvae were exposed acutely to hypoosmotic stress (10‰ S). Metabolic responses of premetamorphic larvae to hyperosmotic stress (30‰ S) were also modified by sublethal fenvalerate exposure. Alterations in metabolic–salinity patterns of larval grass shrimp developing under sublethal concentrations of fenvalerate suggest reduction in the ecological fitness of this sensitive life stage by limiting their capacity to adapt to the fluctuating salinity conditions of estuarine waters.

Key words: fenvalerate; *Palaemonetes pugio*; larval development; oxygen consumption; osmotic stress; pyrethroid insecticides.

INTRODUCTION

In the last decade, environmental concerns associated with pesticide use in the agricultural industry have resulted in changing manufacture and use patterns of insecticides in the United States. Highly persistent and bioaccumulable organochlorine insecticides were initially replaced by organophosphate insecticides, while more recently, synthetic pyrethroid insecticides have been introduced. One such synthetic pyrethroid is fenvalerate, a highly potent and broad spectrum insecticide (Elliott, 1977; Casida, 1980).

There is a need to assess the relative environmental hazards of these potential contaminants to aquatic ecosystems. A review (Khan, 1983), based primarily on freshwater data, suggests that certain methods of applying pyrethroid insecticides

may threaten the fisheries and productivity of aquatic systems. Estuarine organisms are of primary importance in assessing the potential threats of these pesticides to the marine environment; they presumably experience greater exposure to agricultural pesticides which, after application throughout regional drainage basins, collect and concentrate in estuarine waters. Recent laboratory studies have shown the photostable pyrethroid insecticide, fenvalerate, to be extremely toxic to estuarine organisms (Schimmel et al., 1983) and have demonstrated structural disruptions in estuarine benthic communities with low-level fenvalerate contamination (Tagatz and Ivey, 1981). Crustaceans were the most sensitive organisms to fenvalerate exposure in both studies.

A thorough assessment of the potential environment risks of a substance should include examination of chronic, low-level effects on the ecological fitness of the more sensitive life-stages of dominant members of the community. The grass shrimp, *Palaemonetes pugio*, is a ubiquitous estuarine decapod which maintains a dominant role in the energy cycles of tidal marsh estuaries along the eastern and southern coasts of the U.S.A. (Williams, 1965; Welsh, 1975). Since larvae of estuarine crustaceans are particularly sensitive to pesticide exposure (for recent reviews see Williams and Duke, 1979; Costlow, 1982), one of the objectives of this study was to examine the effects of continuous fenvalerate exposure on the complete larval development of *P. pugio*.

Osmotic stress decreases the larval resistance of several crustaceans to toxicant exposure (Vernberg et al., 1973, 1977; Rosenberg and Costlow, 1976; Middaugn and Floyd, 1978; McKenney and Neff, 1979; McKenney and Costlow, 1981, 1982). Sublethal toxicant exposure throughout the larval development of *P. pugio* severely restricts the normally broad salinity range for successful completion of metamorphosis for this euryhaline species (McKenney and Neff, 1979).

Tolerance of osmotic stress is the most essential adaptation required for a population to succeed in the fluctuating salinity conditions of an estuary (for reviews see Lockwood, 1976; Gilles and Jeuniaux, 1979). Osmoregulatory capabilities of palaemonid shrimp are related to the respiratory metabolism of larvae exposed to osmotic stress (Moreira et al., 1982). Moreover, metabolic compensation to salinity by developing *P. pugio* larvae may be modified by sublethal toxicant exposure (McKenney and Neff, 1981). Therefore, to indicate the ecological fitness of larval *P. pugio*, the second objective of this study was to measure larval metabolism during osmotic stress to determine if the ability of larvae to adapt to fluctuating salinities was altered by sublethal exposure to fenvalerate.

MATERIALS AND METHODS

To obtain larvae for the study, adult grass shrimp (*P. pugio*) were collected in shallow grass beds of Santa Rosa Sound, FL, and held in the laboratory in flowing, filtered (20 μm) sea water at 25 ± 1°C and 20 ± 2‰ salinity. Larval grass shrimp were

collected from this laboratory population of adult shrimp by using a flow-through hatching apparatus described by Tyler-Schroeder (1978).

Newly released *P. pugio* larvae were randomly distributed in replicate beakers (10 larvae per beaker) contained within separate exposure tanks for each exposure concentration of fenvalerate. Replicates consisted of 1000 ml glass beakers with a 25 mm diameter hole covered by a 360 μm mesh screen on the side near the bottom of the beaker. Each exposure tank continually received sea water ($25 \pm 1°C$, $20 \pm 1‰$ salinity) containing equal portions of the carrier (triethylene glycol) and nominal fenvalerate concentrations of either 0.0 (the carrier control), 0.1, 0.2, 0.4, 0.8, 1.6 or 3.2 ng fenvalerate/l.

For each exposure tank, a fenvalerate stock solution prepared in triethylene glycol was injected directly by a syringe pump into flowing sea water siphoned from a head trough (Fig. 1). A mixture of fenvalerate, triethylene glycol, and sea water was produced by the cascading movement through the mixing tube and was delivered directly to the exposure tank. An automatic siphon attached to the drain produced fluctuating water levels in the exposure tanks, causing sea water to enter and exit the beakers through the screened holes.

Nominal fenvalerate concentratons were based upon measured fenvalerate stock solutions, syringe pump injection rates, and flow rates of sea water into each exposure tank. Sea-water flow rates and fenvalerate injection rates were monitored daily and maintained within 10% of theoretical rates. Since all nominal concentrations of fenvalerate used during this study were below the detectable limits of established methods, development of a method for the analysis of fenvalerate at low concentrations in sea water by capillary column gas chromatography (detection

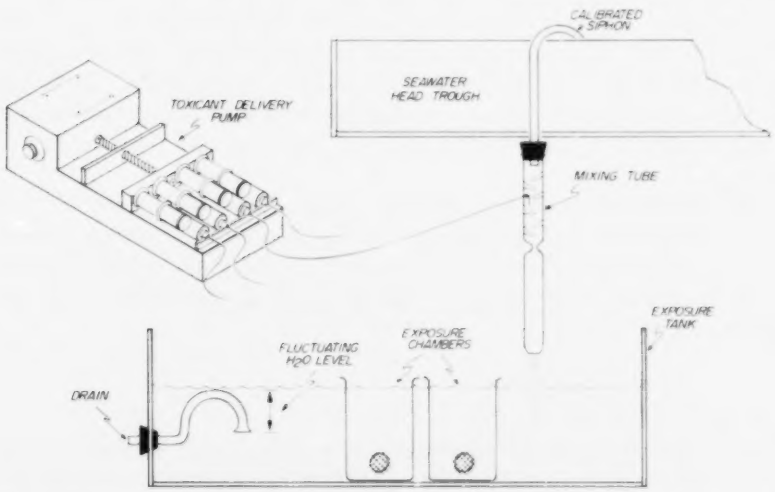

Fig. 1. Exposure system used to expose *P. pugio* through complete larval development to fenvalerate.

limit, 0.02 ng fenvalerate/l) was initiated during the course of this study (Schoor and McKenney, 1983). Throughout the exposure period, the highest exposure level of fenvalerate (3.2 ng/l) was measured weekly.

Larvae within the various beakers were observed daily, at which time they were fed an abundance of freshly hatched *Artemia* nauplii. Positioning of beakers within exposure tanks was systematically shifted on a daily basis to avoid a bias on exposure conditions by location of beaker. Daily records were kept on mortality of larvae in five replicate beakers and the time for development through completion of metamorphosis to postlarva.

Each exposure tank contained additional beakers of larvae which were subsampled for metabolic measurements at various stages of development. One day after larvae were exposed to fenvalerate, larval oxygen consumption was determined at the optimal rearing salinity of 20‰ (McKenney and Neff, 1979) for larvae from all exposure concentrations of fenvalerate. In addition, on days 1, 8, and 16 of larval development, oxygen consumption of larvae from the control and two sublethal concentrations of fenvalerate (0.1 and 0.2 ng/l) was measued at the optimal salinity and at stressful low and high salinities of 10 and 30‰. These salinities were prepared by addition of synthetic sea salts or dilution with distilled water. Similar metabolic–salinity measurements were determined for postlarvae following completion of metamorphosis.

Oxygen consumption rates of individual larvae were determined for seven larvae under each experimental condition using techniques described in detail by McKenney and Neff (1981). Briefly, 5 ml glass syringes, serving as respirometers for individual larvae, were incubated for 4 to 6 h in a water bath at $25 \pm 1°C$. Partial pressures of oxygen in the syringes were determined using the oxygen electrode of a Radiometer BMS3 Mk2 Acid-Base Analyzer. After drying for 48 h at 60°C, dry weights of individual larvae were determined to the nearest 0.1 μg on a Cahn Automatic Electrobalance. Weight-specific oxygen consumption rates ($\mu g\ O_2 \cdot mg^{-1}$ dry wt $\cdot h^{-1}$) were determined using these dry weights and the differences in partial pressures of oxygen in syringes containing larvae and control syringes without larvae.

Values for survival, developmental durations, and oxygen consumption rates are presented as means $\pm$ 1 SE. Significant differences in responses between control and exposed larvae and between experimental salinity conditions were determined by analysis of variance, using Duncan's procedure for multiple comparisons between means (Zar, 1974).

RESULTS

Measured concentrations of the highest level of fenvalerate (3.2 ng/l) during the course of this study varied from 10 to 40% of the nominal concentration dependent on location within the exposure tank (Schoor and McKenney, 1983). For conve-

nience, fenvalerate concentrations used throughout this paper, unless otherwise noted, represented nominal concentrations.

Exposure to nominal fenvalerate concentrations ranging from 0.1 to 3.2 ng/l throughout the complete larval development of *P. pugio* produced differential survival patterns for developing larvae (Table I). However, these concentrations had no significant effect on viablity of grass shrimp larvae until the fourth day of exposure, when 3.2 ng/l produced significant mortality. Only this highest concentration produced a significant reduction in survival of larvae throughout the completion of metamorphosis (Fig. 2).

Continual exposure to fenvalerate concentrations higher than 0.8 ng/l significant-

TABLE I

Percent survival (mean ± SE) of developing *P. pugio* larvae continually exposed to fenvalerate.

Fenvalerate (ng/l)	Day				
	4	8	12	16	20
0.0	94 ± 3	84 ± 5	69 ± 9	61 ± 10	57 ± 8
0.1	96 ± 4	90 ± 5	74 ± 10	70 ± 13	58 ± 11
0.2	95 ± 3	85 ± 6	75 ± 9	70 ± 9	55 ± 16
0.4	90 ± 3	80 ± 3	76 ± 4	62 ± 10	48 ± 12
0.8	100 ± 0	94 ± 2	82 ± 4	74 ± 2	66 ± 2
1.6	90 ± 4	86 ± 6	76 ± 6	74 ± 5	66 ± 5
3.2	47[a] ± 7	39[a] ± 7	34[a] ± 9	32[a] ± 8	30 ± 9

[a]Significantly different from the control (0.0) ($P<0.05$).

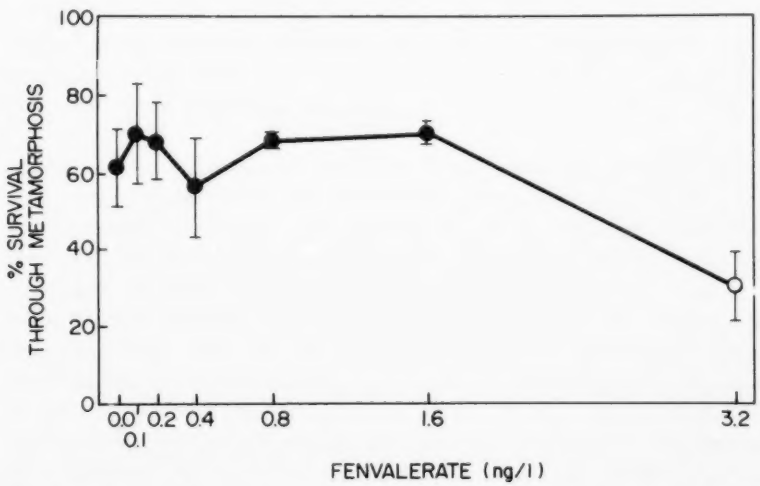

Fig. 2. Survival of *P. pugio* exposed through complete larval development to fenvalerate. Value represented by open circle is significantly different from control (0.0) values.

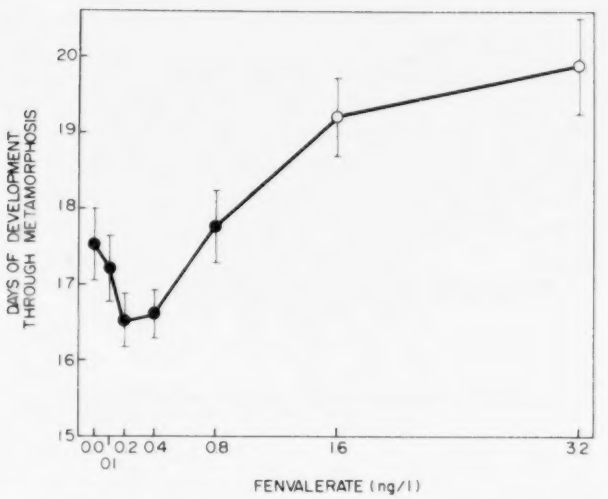

Fig. 3. Developmental duration of *P. pugio* exposed from hatch through completion of metamorphosis to fenvalerate. Values represented by open circles are significantly different from control (0.0) values.

ly prolonged development of larval grass shrimp through metamorphosis in generally a linear fashion (Fig. 3). Larval exposure to 1.6 ng fenvalerate/l delayed the completion of metamorphosis by 1.7 days; exposure to 3.2 ng/l delayed completion of larval development by 2.4 days.

Oxygen consumption rates of *P. pugio* larvae were modified after only 24 hours of exposure to fenvalerate (Fig. 4). They were elevated by exposure to all concentratons, most noticeably by those higher than 0.8 ng/l. However, only exposure to 3.2 ng fenvalerate/l for 24 h produced a significant increase in larval metabolic rates.

Acute exposure to low and high salinities modified the oxygen consumption rates of larval *P. pugio* reared in sublethal concentrations of fenvalerate, but these modifications were dependent on stage of development. Sublethal exposure to fenvalerate concentrations of 0.1 and 0.2 ng/l produced no significant effect on oxygen uptake rates of 1-day-old larvae, even with acute exposure to salinities 10‰ above or below the optimal rearing salinity of 20‰ (Fig. 5A). A significant interaction between fenvalerate exposure and acute salinity stress altered oxygen consumption of intermediate aged *P. pugio* larvae. Following 8 days of exposure to sublethal fenvalerate concentrations (0.1 and 0.2 ng/l), larvae had significantly higher rates of oxygen consumption when acutely exposed to low salinity stress (Fig. 5B). Sublethal exposure to 0.2 ng fenvalerate/l not only significantly lowered rates of oxygen uptake by premetamorphic larvae, but also modified their response to osmotic stress (Fig. 5C). Those premetamorphic larvae exposed to 0.2 ng/l, like 8-day-old larvae,

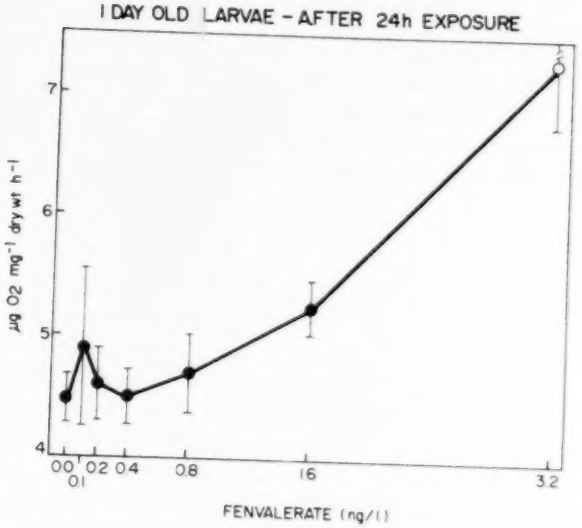

Fig. 4. Oxygen consumption rates of *P. pugio* larvae exposed from hatch for 24 h to various fenvalerate concentrations. Value represented by open circle is significantly different from control (0.0) values.

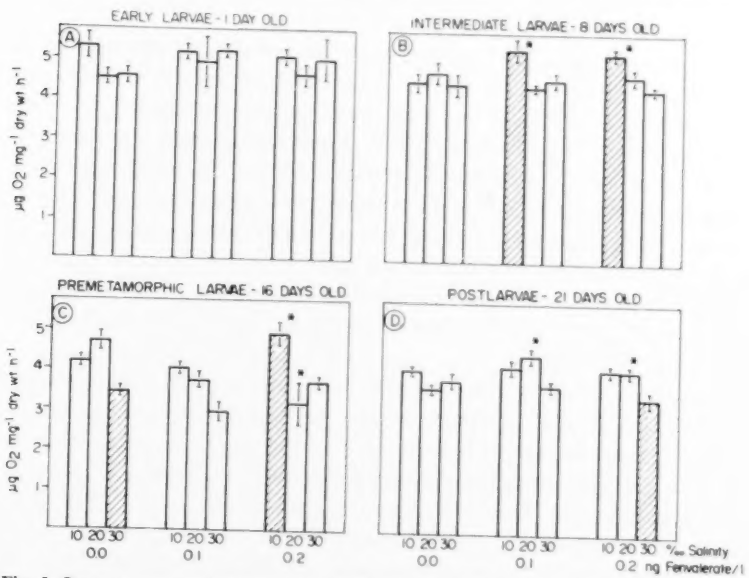

Fig. 5. Oxygen consumption rates of *P. pugio* larvae as affected by acute salinity stress after continuous exposure to fenvalerate from day of hatch. Asterisks denote significant differences by fenvalerate concentration at the same salinity. Shaded bars represent significant differences between salinities at the same fenvalerate concentration.

had significantly higher oxygen consumption rates when acutely exposed to 10‰ salinity, but were unaffected by high salinity stress. Postlarvae, which had been reared through completion of metamorphosis in these sublethal concentrations of fenvalerate, exhibited higher rates of oxygen uptake under conditions of optimal salinity (Fig. 5D). Furthermore, after being reared in 0.2 ng fenvalerate/l, postlarvae had depressed metabolic rates under high salinity stress.

DISCUSSION

Concentrations of fenvalerate that were toxic to larval *P. pugio* represent the lowest levels of fenvalerate that have been reported to produce toxic effects on nontarget aquatic organisms. Greater than 50% of the *P. pugio* larvae exposed to measured concentrations averaging 0.8 ng/l of fenvalerate died within 4 days of continuous exposure. Other toxicity values for fenvalerate range from 22 to 200,000 ng/l for freshwater organisms (Mulla et al., 1978; Coats and O'Donnell-Jeffery, 1979; Anderson, 1982) and from 8 to 5400 ng/l for marine organisms (McLeese et al., 1980; Tagatz and Ivey, 1981; Schimmel et al., 1983). These studies demonstrated that crustacea are more sensitive to fenvalerate than other aquatic organisms, which agrees with the extreme sensitivity of grass shrimp larvae reported in this paper and is consistent with the phylogenetic relationship between crustacea and the arthropod pests for which pesticides are directed.

Direct lethality of fenvalerate to *P. pugio* larvae did not vary significantly from that observed after only 4 days of exposure of early larval stages and that produced with exposure throughout the complete larval period of approximately 3 wk. This mortality pattern during larval development of fenvalerate-exposed *P. pugio* and the observation that no mortality occurred for adult *P. pugio* exposed to these same fenvalerate concentrations (D.B. Hamaker, unpubl. obs.) suggest that early larval stages are the most sensitive period to fenvalerate toxicity during the life cycle of this species. Early larval stages of a number of other estuarine decapod crustacea have been shown to be more sensitive to pesticide toxicity than later life stages (Buchanan et al., 1970; Epifanio, 1971; Armstrong et al., 1976; Christiansen et al., 1977a,b; Caldwell et al., 1978).

Continual exposure to the highest sublethal concentration of fenvalerate (a nominal concentration of 1.6 ng/l) delayed completion of metamorphosis for developing grass shrimp larvae by nearly 2 days. The complete developmental period for larvae of estuarine decapods has been shown to be prolonged by sublethal exposure to various other pesticides (Epifanio, 1971; Bookhout et al., 1972, 1976, 1980; Bookhout and Monroe, 1977; Christiansen et al., 1977a,b), including a delay in the larval development of *P. pugio* with exposure to sublethal concentrations of the polychlorinated biphenyl, Arochlor 1254 (Roesijadi et al., 1976). Extension of the particularly vulnerable pelagic phase in the life cycle may increase predation pressure on the species. Increased predation on larvae would reduce the number

available for recruitment into the parental benthic population or for dispersion and establishment of new populations in less severe environments (Mileikovsky, 1971; Sandifer, 1975).

Sublethal concentrations of contaminants can disrupt normal physiological processes of individual organisms to the extent that the ecological fitness or continued prosperity of the population is impaired (Waldichuk, 1979; Kinne, 1980; Sastry and Miller, 1981). Since an individual's metabolism is an integrative characteristic, a general indicator of metabolic rate such as oxygen consumption rate may be useful in assessing the physiological state or well-being of an organism and, ultimately, serve as a predictor of functional disruptions of the population. Oxygen consumption rates of newly released *P. pugio* larvae were significantly higher after exposure for 24 h to fenvalerate concentrations which, after continual exposure, resulted in significantly fewer larvae completing metamorphosis. Although the molecular mode of action of pyrethroids remains unclear, the toxic action is believed to result from interference with neural transmission (Casida, 1980; Narahashi, 1983; Van den Bercken and Vijverberg, 1983), resulting in hyperactivity. Furthermore, inhibition of mitochondrial ATPase by fenvalerate has been reported (El-Sebae et al., 1981). Therefore, increases in metabolic rates of larval *P. pugio* exposed to fenvalerate may be due to increased nerve or muscle activity and higher rates of metabolism of these tissues.

Other studies have shown disruptions in oxidative metabolism of marine crustacea with pesticide exposure. *P. pugio* exposed to Arochlor 1254 exhibited elevated respiration rates nearly four times that of unexposed controls (Anderson et al., 1974). Subacute internal levels of mirex and DDT caused higher metabolic rates in juvenile blue crabs (Leffler, 1975). Juvenile mysids, *Mysidopsis bahia*, had significantly higher rates of oxygen consumption upon initial exposure to pesticide concentrations which eventually proved lethal (McKenney, 1982; 1984). These results suggest that altered rates of respiration of marine crustacea may serve as a rapid biological monitor of the effects of pesticide exposure to important components of the marine community.

Upon completion of metamorphosis, postlarval *P. pugio* had significantly higher rates of oxygen consumption in sublethal concentrations of fenvalerate, which previously had not altered larval metabolism. Several explanations appear plausible for the elevated metabolic rates of these young juveniles. The dramatic internal reorganizations accompanying completion of metamorphosis may render the overall metabolism of postlarvae particularly sensitive to sublethal fenvalerate exposure. With completion of metamorphosis, this species shifts from a free-swimming pelagic larvae to a benthic postlarvae. The highly adsorptive nature of fenvalerate (Khan, 1983; Schoor and McKenney, 1983) could have resulted in adsorption onto the glass surface of the exposure beakers during this study. Benthic postlarvae would be more exposed to fenvalerate adsorbed to the glass surface than would those in the water column, affording greater bioavailability and increased metabolic sensitivity.

Acute exposure to osmotic stress modified the larval metabolism of *P. pugio* reared in sublethal concentrations of fenvalerate and the metabolic response varied with the stage of development. Elevated metabolic rates of fenvalerate-exposed intermediate and premetamorphic larval forms, when subjected to hypoosmotic stress, suggest a greater energetic cost for these larvae when adjusting to low salinity. The metabolism of prematomorphic larvae was reduced with hyperosmotic stress and these apparent metabolic adjustments to high salinity were modified by sublethal fenvalerate exposure. These ontogenetic variations in larval metabolism, as influenced by salinity and modified by sublethal toxicant exposure, agree with previous observations for *P. pugio* larvae developing in sublethal concentrations of zinc (McKenney and Neff, 1981). Alterations in the metabolic–salinity patterns of larvae with sublethal exposure to fenvalerate suggest reductions in the ecological fitness of these sensitive larval forms by limiting their capacity to adapt to salinity fluctuations in estuarine waters.

Similar mechanisms may be responsible for fenvalerate toxicity and sublethal alterations in larval metabolism of *P. pugio* under osmotic stress. As previously mentioned, pyrethroid toxicity is believed to result from interference with ion transport in nervous tissue in a manner similar to the mode of action of DDT-like organochlorinated compounds (Narahashi, 1983; Van den Bercken and Vijverberg, 1983). In addition, it has been postulated that inhibition of ATPase by pesticides results in altered ion transport processes in the cell membrane and disrupted osmoregulatory abilities of aquatic animals (Bouquegneau and Gilles, 1979). While salinity adaptation of the blue crab, *Callinectes sapidus*, was accompanied by increased gill Na, K-ATPase activity (Neufeld et al., 1980), DDT exposure inhibited the activity of this enzyme in blue crab gills (Neufeld and Pritchard, 1979). Similarly, crabs that were exposed sublethally to the organochlorine pesticide, methoxychlor, had decreased resistance to low salinity stress and partial inhibition of Na, K-ATPase activity (Caldwell, 1974). Less efficient osmoregulatory abilities by larval *P. pugio* would result in additional energy demands with salinity stress. Thus, similar mechanisms of interference with ion transport processes that were responsible for fenvalerate toxicity to grass shrimp larvae may be, at least, indirectly responsible for sublethal alterations in larval metabolism under osmotic stress.

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